The presence of EGFR mutations was also determined in archival tumor material and/or plasma obtained from 39 consenting advanced stage NSCLC patients treated with EGFR-TKIs.

Results: In cell line experiments, mutant DNA was detectable utilizing the Scorpion technology at concentrations as low as 25 pg, and at ratios as little as 0.1% of the total pool of genomic DNA. Of the 39 NSCLC patients, only tissue was available for evaluation in seven patients; two were positive both with the Scorpion primers and direct sequencing while the rest were wild-type. For 21 patients where only plasma was available, 6 mutations were detected with Scorpion primers, none of which were detectable by direct sequencing. EGFR mutations were identified in both plasma and tissue of two patients who were complete responders to EGFR-TKI therapy, only one of which was detectable by direct sequencing. Two additional mutations were found in the tissue but not plasma of patients currently undergoing treatment. Neither of these mutations was detectable by direct sequencing.

Conclusions: Allele-specific Scorpion technology is: 1) highly specific and sensitive for EGFR mutation analysis, 2) able to detect mutations not observable by direct sequencing in plasma and tissue, 3) capable of detecting mutations in shed tumor DNA in plasma and 4) may be suitable for monitoring response or detecting recurrence in advanced NSCLC patients.

B7-05 **BSTB: Molecular Diagnostics & Pathology, Tue, 13:45 - 15:30**

Epidermal Growth Factor Receptor Mutation Analysis by Endobronchial Ultrasound Guided Transbronchial Needle Aspiration

Nakajima, Takahiro[1] Yasufuku, Kazuhiro[1] Suzuki, Makoto[1] Hiroshima, Kenzo[2] Mohamed, Sherif[1] Miyagi, Yohei[3] Matsukuma, Shoichi[3] Sekine, Yasuo[1] Shibuya, Kiyoshi[1] Fujisawa, Takehiko[1]

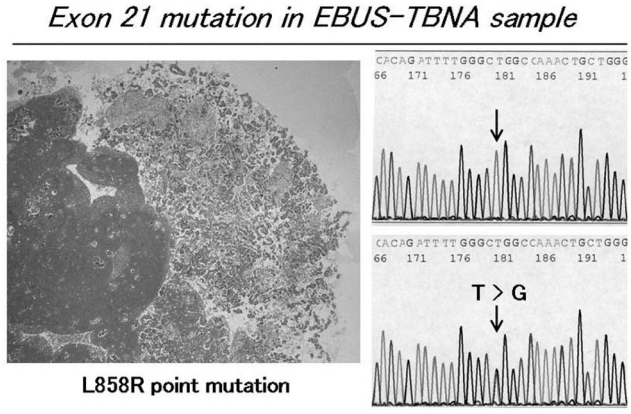
[1] Department of Thoracic Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan [2] Department of Diagnostic Pathology, Graduate School of Medicine, Chiba University, Chiba, Japan [3] Kanagawa Cancer Center Research Institute, Yokohama, Japan

Background: The presence of somatic mutations in epidermal growth factor receptor (EGFR) predicts the response of EGFR tyrosine kinase inhibitors (TKIs). It has been reported that exon 19 and 21 mutation correlates with the effectiveness and exon 20 mutation correlates with the resistance of EGFR-TKIs. It would be ideal if EGFR mutation can be detected by biopsy samples, since the majority of non-small cell lung cancer patients at the time of presentation are inoperable. We have reported the usefulness of endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA). EBUS-TBNA has a high sensitivity as well as specificity for mediastinal and hilar lymph node staging in patients with lung cancer. EBUS-TBNA is a real-time procedure that enables multiple biopsies with good histological cores.

Methods: The purpose of this study was to develop and analyze the feasibility of detecting EGFR mutation in samples obtained by EBUS-TBNA. Fifty six patients with primary lung cancer diagnosed as metastatic adenocarcinoma in hilar and/or mediastinal lymph node by EBUS-TBNA were analyzed. We extracted DNA from paraffin embedded samples and investigated the EGFR mutation in exon 19 and 21 using a newly developed Loop- Hybrid Mobility Shift Assay (LH-MSA). The results were confirmed by direct sequence method in the first 46 cases. Furthermore we analyzed the exon 19 to 21 in 10 cases using LH-MSA.

Results: Out of the 56 cases, 53 cases contained tumor cells in re-sliced paraffin embedded specimens and were analyzed. Thirty four patients (64.2%) were male. EGFR mutation was detected in 15 cases (30.2%); three cases were in-flame deletion of exon 19, one case was point mutation of exon 20, and 12 cases were point mutation of exon 21. 52.6% of the female population (10 out of 19 cases) showed EGFR mutation. Two cases with exon 21 point mutation were treated by gefitinib. A case with multiple mediastinal lymph node metastases along with malignant pericardial effusion showed a significant decrease in size of the tumor two weeks after the administration of gefitinib and has been alive for 22 months.

Conclusions: EGFR mutation can easily be detected in metastatic lymph nodes sampled by EBUS-TBNA. EBUS-TBNA allows genetic evaluations of tumor cells within the lymph node and may provide us with indications for EGFR-TKI therapy before administration of EGFR-TKIs therapy in the near future. EBUS-TBNA samples will possibly provide other molecular biological information which will be useful for the treatment of advanced and recurrent lung cancer disease



B7-06 **BSTB: Molecular Diagnostics & Pathology, Tue, 13:45 - 15:30**

Mass Spectrometry Profiling of Low Molecular Weight Platelet Proteome for the Detection of Lung Cancer Specific Biomarkers

De Petris, Luigi[1] Pernemalm, Maria[1] Brandèn, Eva[2] Koyi, Hirsh[2] Forshed, Jenny[1] Sundelin, Birgitta[1] Lewensohn, Rolf[1] Lehtiö, Janne[1]

[1] Karolinska Biomics Center, Karolinska Institutet, Stockholm, Sweden [2] Dept. of Lung Medicine, Karolinska University Hospital, Stockholm, Sweden

Background: In preclinical models, proteomic profiling has revealed that circulating platelets selectively take up tumor-derived angiogenesis regulatory proteins (J. Folkman et al. FASEB J 20:A414,2006). The aim of this study was to assess whether this novel function of platelets could be efficiently explored with proteomic profiling of the low molecular weight (LMW) proteome, and further reveal lung cancer (LC) specific markers.

Methods: Blood samples (4-ml EDTA tubes) were collected from 54 patients (29 with newly diagnosed advanced NSCLC and 25 with benign lung diseases) under the same conditions. Written informed consent was obtained from all patients. LC patients included 18 adenocarcinomas, 7 squamous tumors, 2 large cell carcinomas and 2 low differentiated NSCLC. The control group included 5 patients with hamartoma, 14 with non-specific lung infiltrate and 6 with diverse be-

nign conditions. The two patient groups were matched with respect to gender, age, smoking habits, number and class of current medications. Patients with benign diseases had a higher median number of co-morbidities compared to LC patients (2 vs 1; p<0.01). As expected, mean platelet concentration in the blood samples was higher in the LC group compared with the control group (370,000/µl vs 292,000/µl, p=0.01). Within 2 hours after blood sample collection, platelets were isolated from plasma using a separation protocol based on sequential centrifugations. Protein concentration was normalized to 2 g/l. Platelet lysates were profiled in duplicates on a strong anionic exchange (Q10, pH 7.5) ProteinChip array, using SELDI-TOF-MS technology. Analyzed mass range was 2,600-20,000 Da. Peaks were detected and quantified using our newly developed method based on 'regions of significance' (C.S. Tan et al. Proteomics 6:6124,2006). Differences in median relative peak intensity between groups were assessed using the Mann-Whitney non parametric test. Significantly different peaks at this univariate analysis were considered potential biomarkers. Multivariate analysis was performed using the supervised partial least square discriminant analysis (PLS-DA).

Results: Spectra showed a very high homology (mean peak intensity CV=46%) despite the expected inter-individual variability, indicating the good reproducibility of the sample preparation method. Overall, approximately 200 peaks were detected. 7 unique peaks were significantly more expressed in LC group compared to the control group (p<0.05). Differences in protein expression due to drugs affecting platelet function, such as low dose acetylsalicylic acid, and drugs taken by at least 20% of patients, were also investigated. 5 and 37 unique peaks were significantly related to the administration of acetylsalicylic acid and inhalation adrenergics, respectively. Furthermore, the PLS-DA provided an initial assessment of the most important discriminant variables that in combination were able to separate LC patients from the control group, although the prediction properties of this model are still to be elucidated.

Conclusions: The preliminary data from this pilot study confirm the hypothesis that the platelet LMW proteome can be a source of biomarkers of systemic diseases, such as advanced LC, that are not directly related to platelet function.

B7-07 **BSTB: Molecular Diagnostics & Pathology, Tue, 13:45 - 15:30**

Deletion of chromosome 10q detected by Fluorescent In Situ Hybridization (FISH) is a potential new tool for early detection of Non Small Cell Lung Cancer (NSCLC)

Yendamuri, Sai; Vaporciyan, Ara A.; Zaidi, Tanweer; Fernandez, Ricardo; Rice David C.; Walsh, Garrett L.; Swisher, Stephen G.; Spitz, Margaret; Roth, Jack A.; Katz, Ruth L.

University of Texas MD Anderson Cancer Center, Houston, TX, USA

Introduction: Deletions in the 10q region are a relatively common and early finding in NSCLC. FISH is a reproducible and accurate technique of detecting these deletions in clinical specimens. This study looks at 10q deletions detected by FISH in different regions of the airway in patients undergoing surgical resection of NSCLC in an effort to determine the feasibility of using FISH for early diagnosis of lung cancer.

Methods: FISH was performed on specimens from 120 patients with probes developed in house on specimens from five different areas:

1 NBB:Brush biopsy from bronchoscopically normal mainstem bronchus from the side opposite the tumor
2 TBB:Brush biopsy from bronchoscopically normal mainstem bronchus from the side of the tumor
3 TAB:Touch preparation from normal bronchus adjacent to the tumor
4 TTP:Touch preparation from the tumor
5 NTP:Touch preparation from normal lung away from the tumor

A FISH assay using probes complementary to 10q22.3 and the centromere of chromosome 10 were used. 10q was considered to be deleted if there were fewer 10q signals when compared to centromeric 10 signals. The Wilcoxon signed rank test was used to compare the ratio of the percentage of deletions detected at different anatomic sites. Correlations of deletions at different sites were estimated using a Spearman correlation coefficient.

Results: Contralateral normal bronchial tissue (NBB) showed a relatively low deletion rate at 10q compared to tumor tissue (TTP) (p<0.0001). FISH on brush biopsies at TBB showed a significantly higher rate of deletions compared to NBB but lower than TTP from the tumor (p<0.05). A significantly higher deletion rate was seen at TTP compared to NTP at the 10 q regions (p<0.0001) (Figure 1). Significant correlations were seen between the rate of deletions between TTP and TAB at 10q (σ=0.64, p<0.0001).

Conclusion: 10q chromosomal deletions can be reliably detected by FISH in bronchoscopic brush biopsies and tumor touch preparations. As one progresses from area of the lung and bronchus away from the tumor to areas proximate to the tumor, the 10q deletion rate increases in a statistically significant fashion, supporting an etiologic role of these deletions. 10q deletions in bronchoscopically normal areas correlate with increased deletion rate in the tumor itself, suggesting that this test may be useful in early diagnosis of NSCLC.

Figure 1
Distribution of deletions in the airway